OSMOTIC REGULATION AND ADAPTIVE REDUCTION OF WATER-PERMEABILITY IN A BRACKISH-WATER CRAB, RHITHROPANOPEUS HARRISI (BRACHYURA, XANTHIDAE)

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Recent studies by various authors have made it evident that among crustaceans of brackish and fresh waters there exists considerable diversity in the combinations of mechanisms of primary importance in osmotic regulation. This is to be expected because the crustaceans of such waters do not represent a single evolutionary line, but rather are representative of different orders and families, some of whose species have independently adapted to conditions of low salinity. While it is probable that certain physiological mechanisms of adaptation to brackish or fresh water are shared by different crustacean groups, there is no *a priori* reason to assume that all groups emphasize a common set of mechanisms. This has been especially clearly indicated in the work of Shaw (1959, 1961b), who has shown that the adaptation of the African crab *Potamon niloticus* to fresh water takes the form of a lowering of body permeability to both salts and water, with the production of a very small amount of blood-isotonic urine. In contrast, fresh-water crayfishes show a lowering of surface permeability to salts, but not so much so to water; the urine is consequently copious, but salts are actively recovered from the urine, which is rendered hypotonic to the blood. That there is no necessary relationship between a fresh-water habitat and the production of hypotonic urine has been indicated by Lockwood (1961), who has shown hypotonic urine production in both fresh-water and brackish-water amphipods, and by Parry (1957), who has demonstrated that the fresh-water prawn *Palaemonetes antennarius* produces a copious but blood-isotonic urine.

The true crabs (Brachyura) are a basically marine group, from several families of which representative genera and species have independently made physiological adaptations to life in fresh or nearly fresh waters. It would be of interest to know whether or not the extremely low urine production of *Potamon* (Shaw, 1959) represents the culmination of an adaptive trend toward reduction of water-permeability common to, and expressable as a generalization for, those brachyurans entering fresh waters. On the other hand, as pointed out by Potts and Parry (1964, p. 174), the apparent low water-permeability of *Potamon* may represent either a uniquely low water-permeability ($\frac{1}{10}$ that of any other fresh-water crustacean) or it may be the result of extra-renal water excretion. Either of the latter two possibilities would be inconsistent with the generalization that reduced permeability to water is a mechanism utilized by euryhaline or fresh-water Brachyura as a group. The crabs studied by Shaw include *Carcinus* (Portunidae), *Eriocheir* (Grapsidae) and *Potamon* (Potamonidae). Although these are arranged in a

series of increasing adaptedness to low salinity and fresh water, they do not
constitute an actual evolutionary series related by descent within the Brachyura.
Rather, *Eriocheir* is in the grapsoid division of the Brachyrhyncha while *Carcinus*
and *Potamon* are in the cancroid division of that group, and hence are more closely
related to each other than either is to *Eriocheir*. In order better to assess the
possibile generalization that a reduction in water-permeability is a physiological
mechanism common to the cancroid crabs represented by *Carcinus* and *Potamon*
it is of interest to evaluate the osmotic performance of a cancroid crab which, in its
euryhalinity, stands between *Carcinus* and *Potamon*. This paper reports on the
small crab *Rhithropanopeus harrisi* (Gould), which appears to be the best-adapted
to low salinities of any of the Xanthidae, a large family of marine and brackish-water
crabs.

Ecology and Distribution

Rhithropanopeus harrisi has its center of distribution on the central Atlantic
coast of the United States. From there it is thought to have been introduced into
the formerly brackish Zuider Zee of Holland (Buitendijk and Holthuis, 1949)
some time before 1874. Since 1936 it has been recorded from Germany, Denmark,
and southern Russia. In Holland, the form was known prior to 1949 as *Hetero-
panope tridentata* (Maitland), formerly *Pilumnus*, and is given subspecific rank as
Rhithropanopeus harrisi (Gould) *tridentatus* (Maitland) by Buitendijk and
Holthuis (1949). *R. harrisi* has also reached the west coast of the United States,
where it has become established in estuarine waters of low salinity about the San
Francisco Bay estuarine system (Jones, 1941). In this region, as elsewhere,
adults may also be found occasionally in fresh water, but the species does not
appear to be capable of reproducing therein.

Previous studies by Jones (1941) and Kinne and Rotthauwe (1952) have shown
that *Rhithropanopeus harrisi* hyper-regulates in lower salinities (below *ca.* 60%
sea water) and conforms or remains slightly hypertonic to higher salinities. Nothing
is known of the mechanisms involved; hence the following observations on water-
permeability and urine production represent a first assessment of osmoregulatory
mechanisms in the species. Crabs for this study were collected in the Napa River,
an estuary opening into San Francisco Bay, where they occur in numbers under
rocks laid as a protective layer on muddy intertidal banks. Salinities are variable,
being less than 5% of sea water in the rainy winter season, but above 25%
sea water in summer.

As an experimental animal for comparative physiology, *Rhithropanopeus harrisi*
is well suited by virtue of its wide salinity tolerance, abundance, and its extensive
(and increasing) geographical distribution, but for some purposes its small size,
up to 2 cm. across the carapace and rarely exceeding 4 grams in weight, at least
in this area, is a disadvantage. In contrast, *Eriocheir* and *Carcinus* commonly
exceed 100–150 grams, and *Potamon* 20–30 grams.

Methods

Crabs undergoing experimental adaptations were maintained at 13–14° C. and
were fed chopped fish twice weekly. Prior to determination of "adapted" values

for blood and urine, crabs were held at the test salinity at least 5 days; experience has shown that osmoregulatory response is esssentially complete within 2 days in the steps of not over 25% sea water used in experimental changes of salinity.

D_2O determinations in blood samples were by the simplified bromobenzene-kerosene gradient column method (Smith, 1964) using D_2O standards made up in distilled water. Each blood sample was drawn in a sharp capillary by puncture of the arthrodial membrane at a leg-base, and introduced into an "Aloe" disposable pipette (short style) that had been boiled in distilled water and oven-dried. A light plug of cotton was inserted past the constriction, the sample drop placed between the mouth and the constriction, the pipette tightly closed at the base by a small cork, and the tip sealed in a flame. The sealed pipettes were laid on a slide-warmer set at 50° C., with their tips either in air or resting on a chilled brass block, and the samples distilled to dryness overnight. D_2O concentrations could be estimated in the gradient columns to about 0.1% (mean of 2), and were expressed as a percentage of the concentration of D_2O in the bathing media.

Chloride determinations of blood, urine, and media were made with a Buchler-Cotlove chloridometer. Samples were collected in 1- or 2-mm.[3] disposable pipettes (Drummond "microcaps"). For collection of urine, crabs were dried with absorbent paper, and the area around the urinary pores coated with beeswax applied with a warm cautery needle, so that emitted urine did not drain away into the sutures next to the apertures. The opercular plate covering the antennal gland aperture was lifted by a fine hooked needle. The crab in response might or might not emit a drop of clear urine, some of which was collected by capillarity in a microcap. Attempts to catheterize these small crabs (mostly less than 2 grams) have been unsuccessful or resulted in bleeding.

Other methods are described in connection with certain experiments, below.

EXPERIMENTAL

A. *Blood concentration as a function of salinity*

In Figure 1 are plotted data showing the pattern of chloride regulation in the blood of *Rh. harrisi* after adaptation to selected salinities. Also in Figure 1 are plotted the means of a series of determinations of the osmotic pressure of the blood obtained by a student, Miss Etta Kwan, using the comparative melting point method of Gross (1954) with reference to NaCl standards. Since the osmotic pressure of the blood is not entirely the result of its Na^+ and Cl^- concentrations, the osmotic pressure curve lies, as expected, a little above that for chloride. In general, the results of Jones (1941) and Kinne and Rotthauwe (1952) are confirmed, except that our data show a slight hypotonicity of blood of crabs in salinities above *ca.* 70% sea water, whereas the previous authors' figures indicate a slight hypertonicity in that upper salinity range. From about 60% sea water down to nearly fresh water there is a clear state of hyper-osmotic and chloride regulation, and in this range of salinities we may expect to find regulatory mechanisms most active. The level of chloride regulation at low salinities (but above fresh water, in which most crabs become sluggish, and in which a high percentage die after a few days) approximates the value of 242 mM/L. reported by Shaw (1959) for *Potamon niloticus* in fresh water. *Rhithropanopeus* thus behaves as do other Brachyura, maintaining a high
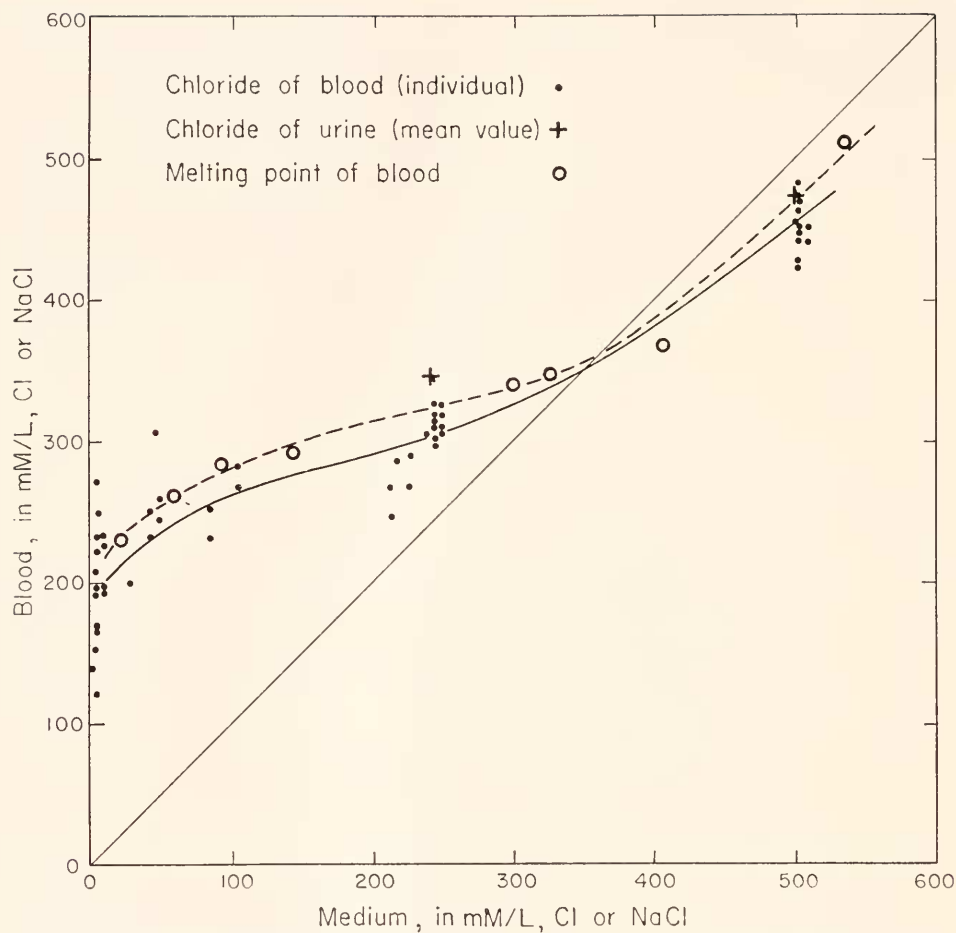
FIGURE 1. Concentration of blood and urine as a function of salinity (expressed in mM of Cl⁻ or NaCl per liter). Circles: melting point of blood expressed as equivalent NaCl concentration, each circle the mean of 8–16 determination on different animals. Dots: Cl⁻ from duplicate samples on individual crabs. Crosses: mean values for urinary Cl⁻, based on 10 of animals furnishing blood values at salinities indicated. Melting point, urinary Cl⁻, and 13 of blood Cl⁻ data obtained by Miss Etta Kwan.

blood concentration, rather than reducing osmotic stress by lowering blood concentration to the degree shown by crayfishes.

B. *The chloride concentration of the urine*

Urine in *Rh. harrisi* is fairly copious. The collection of urine was carried out under a dissecting microscope at 18×, so that any admixture of blood could be readily detected by the observation of blood cells in the sample; any such samples were discarded. Following the obtaining of two urine samples, blood samples were obtained in duplicate by puncture of the arthrodial membrane at a leg base and
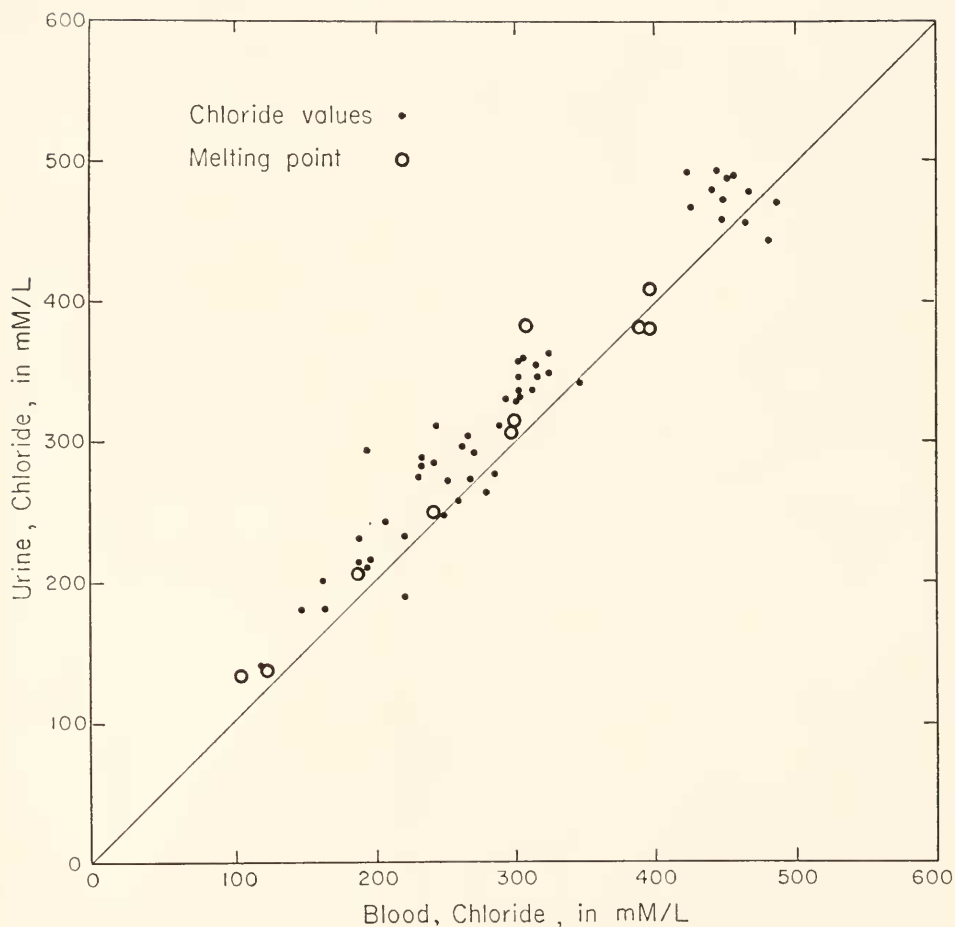
FIGURE 2. Urinary Cl⁻ and osmotic pressure as function of Cl⁻ and O.P. of blood. Each dot based on duplicate blood and urinary Cl⁻ determinations on same animal. Each circle based on freezing point data, expressed as equivalent NaCl, obtained by Miss Etta Kwan and used also in Figure 1.

insertion of a disposable pipette. The results of such paired determinations are shown in Figure 2, in which the values for urine samples (means of two) are plotted against the mean values of the duplicate blood samples. It is clear that the chloride of the urine is slightly greater than that of whole blood. However, since the blood contains proteins, and hence has a lesser water-content than the urine, it is presumed that urine and blood, on water-content basis, are isotonic in respect to chloride. Robertson (1949) has calculated a mean water content of 93.5% by weight in the blood of 3 marine crustacean species. Such a value would essentially account for the difference in chloride concentration between whole blood and urine in *Rh. harrisi,* and in part for the apparent hypotonicity of the blood of animals in sea water. However, since both chloride and osmotic pressure of the

urine are slightly below those of sea water when animals are adapted to that medium, it seems probable that *Rh. harrisi* shows a slight ability to hypo-regulate in sea water, as is common among brackish-water decapod crustaceans. *Rh. harrisi* resembles all other species of crabs so far studied in not having adopted the production of hypotonic urine as an osmoregulatory device. The apparently copious amount of urine suggested a high water intake, and attempts were made to evaluate urinary output and water intake as functions of salinity.

C. *Urine volume and rate of production*

Although urine can usually be readily collected from *Rh. harrisi,* the amounts collectable reflect the storage capacity of the bladders rather than rate of formation. Attempts to cannulate these small crabs have not been successful. Although several workers have estimated urine production in crustaceans by blocking of urinary pores and noting the consequent weight increase, the method has not proved satisfactory. Not only are the pores difficult to block (see below), but it is difficult to dry crabs consistently before weighing, resulting in imprecision. Further, there is the probability that obstructing urinary outflow prevents by back pressure the normal production of urine. Finally, in my experiments, even where urinary blockage was achieved and weight increases noted, no crab survived a weight increase of over 5%. Since such fatal increases took place in 10–20 hours in 10% sea water, one can conclude only that urine production in such a medium exceeds 5–10% of the body weight per day, and that the ability to release urine is of great physiological importance to the animal. Consequently a more complex and tedious but physiologically less drastic method was employed.

Crabs from a given medium (10% or 50% sea water) were individually numbered and weighed (after drying by wrapping in a towel and shaking). For each crab, a beaker of glass-distilled water was prepared, in volume 50 ml. per gram wet weight of crab. A seal of beeswax was applied to each crab with a warm cautery needle, completely covering the orbits and the sockets of antennae and antennules, but leaving the urinary opercula exposed. For each crab a series of three 50-ml. portions of distilled water was set up in small beakers. At timed intervals, each animal was passed through these 3 washes of distilled water to remove external salts, the total time in these preliminary baths being 5 minutes, at the end of which the crab was transferred to the test bath of glass-distilled water, the volume of which was known and in proportion of the animal's weight. After one hour in this first test exposure (A) each crab was returned to the adaptational medium, and a sample of the test medium saved for chloride analysis. A second set of test media (B) of glass-distilled water, of the same volumes as set (A), was then prepared, as well as fresh sets of the three 50-ml. washes. Each crab was then dried off, and the beeswax seal extended to cover its urinary pores. The elaborate sealing procedure was necessitated by the fact that two deep sutures at the sides of the movable antennal base interrupt the rim of each urinary pore and communicate with the areas about the bases of eyestalks, antennae, and antennules; weight increases were not observed in the earlier attempts to estimate urine production by blockage unless the seals were thus extended. Each "fully sealed" crab was then washed through 3 changes of distilled water and exposed for a second hour

TABLE I

Urine volumes and urinary salt losses

Adaptational media	10% SW	50% SW	Pooled
Number of animals	16	15	31
Mean wet weight (grams)	1.82	1.66	1.74
Mean Cl-loss per gram of crab (mM) "A" "B" "C"	.0067 .0049 .0078	.0099 .0075 .0113	— — —
% of Cl lost via urine Range Mean ± S.D.	−4.84 to 87.1 35.7 ± 24.6	−7.14 to 78.2 34.0 ± 23.2	−7.14 to 87.1 34.9 ± 23.5
Urine, % body weight ± S.D. Range (per hour) Mean (per hour) Mean (per day)	−0.62 to 2.17 0.97 ± 0.69 23.3 ± 16.6	−0.30 to 2.07 1.05 ± 0.75 25.2 ± 18.0	−0.62 to 2.17 1.01 ± 0.71 24.2 ± 17.0

(B) to glass-distilled water, at the end of which period it was again returned to its adaptational medium and water samples (B) taken. Next, the wax seal was removed from the urinary opercula of each crab, and a third series (C) of the 50-ml. washing baths and measured proportionate volumes of glass-distilled water prepared. Each crab was washed as before and exposed to distilled water for a third hour (C), at the conclusion of which each crab was removed and samples of the test media taken. Chloride concentrations of all test media (A, B, and C) were determined, and the mM of chloride lost per gram of crab determined. The chloride loss in the second hour (B; urinary pores blocked) was subtracted from the average loss in the first and third hours (A and C; urinary pores open). The difference was taken to represent that part of the total chloride loss which normally occurred *via* the urine. (The probable loss of some chloride through the thin membranes of the occluded orbital and antennal areas has been ignored.) The values obtained (see Table I below) indicate that about one-third of the total chloride loss in the experimental situation is *via* the urine, the fraction not being greater in the crabs adapted to 50% sea water than in those adapted to 10% sea water before the test.

If we may assume that in the above experiment the crabs continued to produce blood-isotonic urine, and that the one-hour exposures, separated as they were by restorative periods in the adaptational media, did not greatly alter the blood concentration, then it should be possible to calculate the volume of urine produced in one hour by using the relationship:

$$\frac{\text{Volume of urine}}{\text{Volume of test medium}} = \frac{\text{Concentration of urinary Cl}^- \text{ in test medium}}{\text{Cl}^- \text{-Concentration of urine}}$$

The above assumptions were tested by exposing a number of crabs (previously adapted to 50% sea water) in a large excess volume of glass-distilled water, changed at intervals, and testing the blood and urinary chloride concentrations of
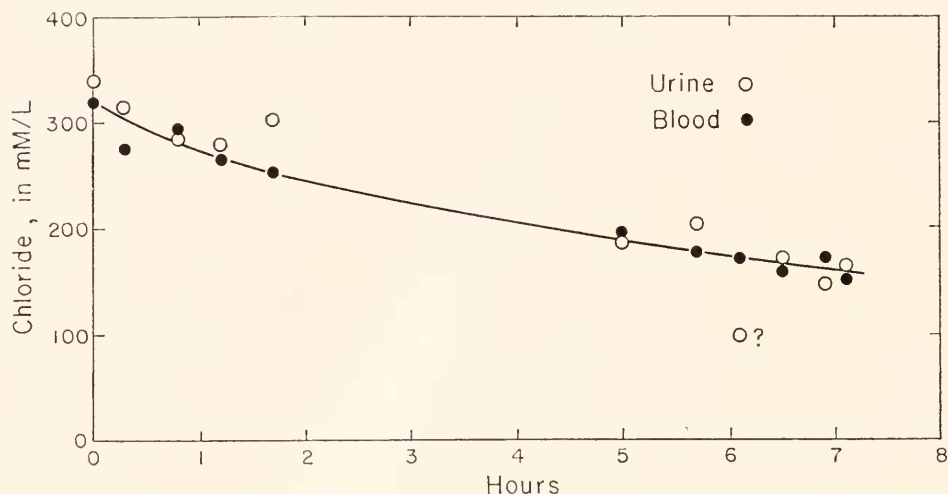
FIGURE 3. Chloride concentration of blood and urine during a 7-hour exposure to distilled water (after 50% SW), each pair of points based on a single individual. Dots, urine; circles, blood. Each point the mean of two samples.

individuals at times up to 7 hours. Figure 3 shows that the urine remains essentially isotonic (blood as usual shows the lesser chloride concentration attributed to its protein content), and that in one hour the drop in chloride concentration is only about 10%. It has therefore been assumed, on the basis of Figures 1 and 3, that blood (and consequently urine) chloride concentrations were 300 mM/L. in crabs adapted to 50% sea water and 250 mM/L. in those from 10% sea water. It is also assumed that the chloride losses in the test exposures were largely made good during the more-than-one-hour periods in the adaptational media which separated test periods A and B from B and C. The results of calculations based on the above assumptions indicate (Table I) that *Rh. harrisi* in the emergency of exposure to fresh water is capable of producing a quantity of urine approximately 1% of its body weight per hour, or 24% per day. Again there is no indication that crabs adapted to 50% sea water eliminate more urine in the test than do crabs from 10% sea water, despite the difference in the osmotic stresses or gradients. Twenty-four per cent of the body weight per day is undoubtedly in excess of that produced in most salinities, and probably tends to exceed the output in the lowest salinities met in nature. Figure 3 indicates that a 50% lowering of the chloride concentration of the blood can occur in 7 hours. This salt loss represents an adaptive lowering of internal osmotic pressure, significantly reducing the stress imposed by externally reduced salinity. It is evident that urine production, while accounting for one-third of the total salt loss, is at the same time of adaptive value in lessening osmotic swelling.

The above conclusions are only general approximations, since the method employed does not exclude several sources of error; *e.g.*, (1) Crabs might discharge urine during handling (observed) or washing prior to exposures A and C, and then might not release urine during these periods. (2) Crabs might release urine during A and/or C which had been stored up prior to the exposure and which rep-
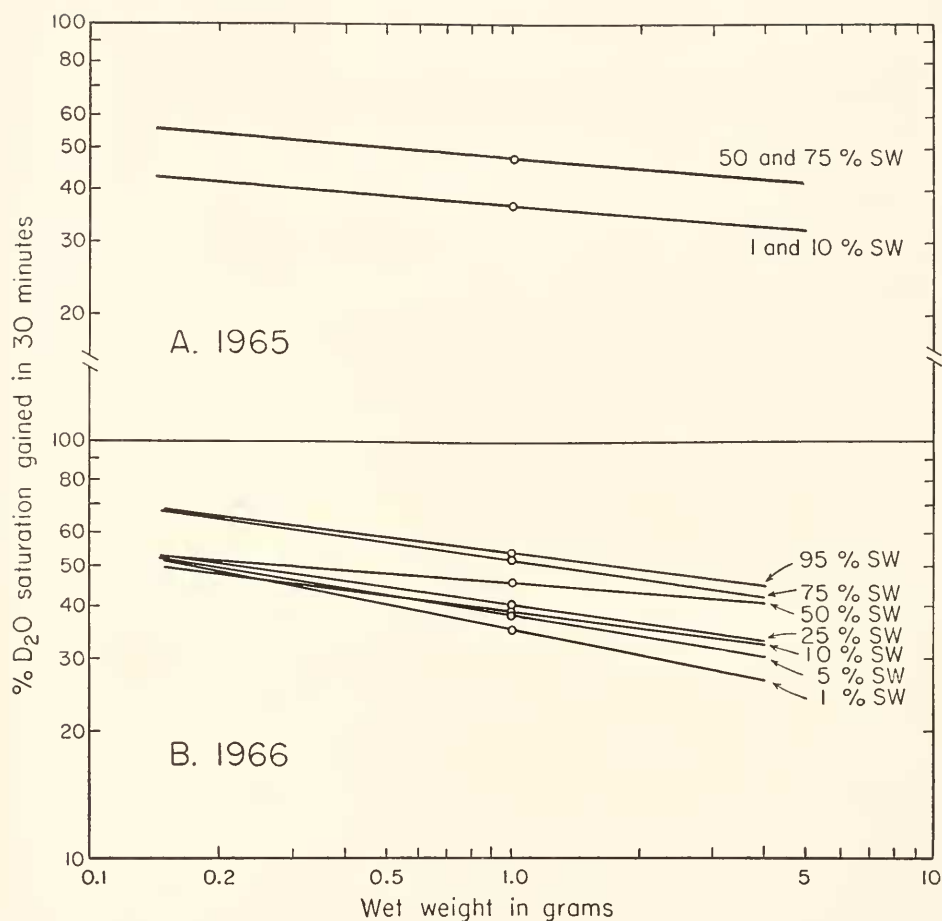
FIGURE 4. D_2O uptake as a function of body weight and salinity (as % sea water); A, 1965; B, 1966. Curves calculated by method of least squares on assumption that: Uptake = a•Weight to power $(b-1)$. Data in Table II.

resented more than an hour's accumulation. (3) Crabs might leak urine from beneath the seal, or might defecate during period B (despite the fact that crabs were not fed for several days prior to these experiments, defecation was observed in a few cases). The great scatter of the data, including even some "negative" values for urine production, suggests that such factors as enumerated above, in addition to experimental error, should dictate caution in accepting the results, but the mean values for urine production are reasonable and quite close to those for the urine of *Carcinus* in 40% sea water (Shaw, 1961a; pp. 144–145), and less than those of fresh-water crustaceans (except *Potamon*) cited by Potts and Parry (1964; p. 175). In one respect the data fail to reveal an expected relationship, namely, the volume of urine produced is not greater in animals adapted to 50% sea water prior to test than in those adapted to 10% sea water, although the greater

osmotic gradient would seem to favor a greater intake and hence greater output of
water in the animals from 50% sea water.

D. *Permeability to D_2O-entry as a function of external salinity*

The data presented above indicate that *Rhithropanopeus harrisi* can produce a
copious urine under osmotic stress, hence under such conditions water must enter
fairly readily.   The methods used, however, do not indicate how much water enters
under more normal conditions and, to determine the permeability of the animal to
inward passage of water, tests have been made of the rate of entry of "heavy water"
(deuterium oxide, D_2O) at various salinities with which the crabs were in steady-
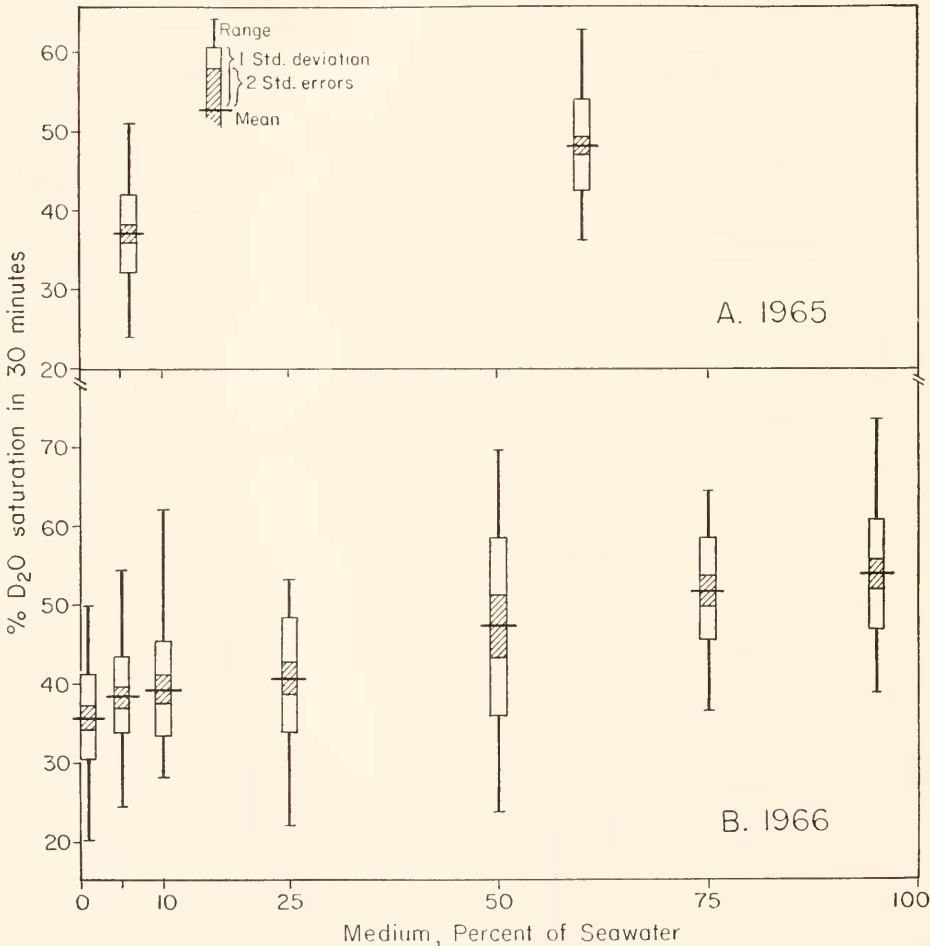

FIGURE 5. D_2O uptake as a function of salinity, corrected to body weight of 1 gram; A,
1965; B, 1966.   Data in Table II.   Plotted by method of Dice and Lerass (1936) (Contr. Lab.
Vert. Genet., Univ. Mich., no. 3: 1–3), in which non-overlap of blocks representing ± 2 std.
errors indicates significant difference.

TABLE II

*D_2O-uptake (as % saturation gained in 30 min.) in relation to salinity (as % SW)
and to body weight*

% Sea water	Fig. 4: % sat = a Weight $^{(b-1)}$			Fig. 5: % sat. corrected to 1 gram weight			
	n	a	(b − 1)	Mean	Std. Dev.	Std. Error	P
1965 series							
1 + 10% SW	77	36.55	−.0802	37.0	± 4.94	±0.56	<.001
50 + 70% SW	92	47.68	−.0725	48.0	± 5.64	±0.59	
1966 series							
1% SW	46	35.32	−.1950	35.8	± 5.39	±0.80	<.025
5% SW	49	38.53	−.1596	38.4	± 4.79	±0.68	>.40
10% SW	47	38.90	−.1292	39.3	± 5.91	±0.86	>.20
25% SW	49	40.25	−.1452	40.9	± 7.20	±1.03	<.005
50% SW	30	45.79	−.0653	47.2	±11.14	±2.04	<.05
75% SW	43	51.88	−.1526	51.8	± 6.44	±0.99	>.10
95% SW	50	53.46	−.1363	53.9	± 7.06	±1.00	

state equilibrium. Preliminary tests showed that D_2O entry proceeded at a uniform rate for at least 3 hours (although a decrease later occurred, suggesting the presence of a second compartment) and that half-saturation of the blood required less than one hour. The method adopted was, therefore, to immerse weighed crabs for one-half hour in 40 times their weight of a 5 moles per cent D_2O solution made up at the salinity to which the crabs had been adapted (thus imposing no osmotic shock). At the end of the half-hour exposure, a blood sample was taken. Since D_2O uptake rate may be closely related to surface in one species, and more nearly proportional to weight in a related species (Smith, 1964), as wide as possible a range of sizes of crabs was employed (0.1 to 4 grams) and the D_2O uptake rate plotted against weight (Fig. 4). Uptake rate is expressed as the per cent of D_2O-saturation obtained in the half-hour exposure to D_2O made up in various percentages of sea water. In the first series of experiments, done in summer and fall of 1965, uptakes of 77 crabs in 1 and 10% sea water were not distinguishable; results were pooled, and the same was done with results obtained on 92 crabs in 50 and 75% sea water. Two conclusions are suggested by the curves in Figure 4A: First, the low slopes (−.0725 and −.0802) indicate that body size (weight) has but a small effect upon uptake rate; absolute uptake is more closely related to body weight than to surface. This means either that the permeability of larger crabs is relatively increased or, more likely, that the permeable areas (such as gills?) show a relative increase with increased body size, such that for purposes of water-entry the surface/volume ratio is kept nearly constant. Secondly, the levels of the curves in Figures 4A and 5A show that the entry of water (as represented by D_2O) is significantly lower (t test) at lower salinities (1–10% sea water, in the regulatory range) than it is at higher salinities (50–75% sea water, in which little if any regulation takes place). This second result was unexpected, since it might be presumed that the osmotic gradient of crabs regulating in low salinities would cause a greater water influx than in crabs in equilibrium with media of higher salinity.

In order to confirm the decrease in water (D_2O) permeability indicated in the 1965 experiments, a more extensive experiment was carefully carried out in the summer of 1966, using a total of 315 crabs from the same locality, adapted at 13° C. to a series of dilutions of sea water (1, 5, 10, 25, 50, 75, 95%), following closely the methods used in 1965. The results, summarized in Table II and plotted in Figure 4B, indicate that the decrease in D_2O permeability with decreased salinity is gradual over the range from 95% to 1% of sea water. The slopes $(b-1)$ of the weight-uptake curves, averaging -0.1404 (-0.0653 to -0.1950), are greater than those obtained in 1965, but are still much less than the value (-0.3333) indicative of the "surface rule," *i.e.*, they still suggest that there is a tendency to compensate for increase in body size by some relatively greater increase in permeable surface. In order to assess the significance of the values obtained, the D_2O uptake rate of each crab was corrected to that of a crab weighing one gram, utilizing the $(b-1)$ values for each salinity group. Figure 5 and Table II show the ranges, standard deviations, and standard errors of the 1965 and 1966 data so corrected. The D_2O uptakes are significantly different at better than the 1% level (t test) between 1%, 25%, 50% and 95% sea water, but the change is gradual enough to be less than significant between most adjacent salinities in the series. The probability that the series as a whole is due to chance is so low that the results in 1966 may be considered highly significant in a statistical sense, but what the significance is in a physiological sense is a more difficult problem.

How a reduction in permeability occurs (or if it occurs) is not clear. Conceivably there could be a reduction in permeable *area* rather than reduction in water permeability *per se*, but it seems more reasonable to seek a physiological mechanism for altering permeability than some morphological change of permeable area. It should be noted that these crabs were adapted to the salinities used in the above experiments for periods of 1–3 weeks, so that the differences are not the result of a long-term ontogenetic conditioning, nor are they consequent upon a molt. However, the minimum time required to effect a measurable water-permeability lowering has not yet been determined.

E. *Water content*

The water content of *Rh. harrisi* was estimated for a group of 15 crabs (wet weights 2.05 to 4.04 g.) adapted to 10% sea water and for a second group of 14 crabs (wet weights 2.05 to 4.14 g.) adapted to 50% sea water. Each group included two females and all animals possessed complete sets of legs and chelipeds. The size range was typical of that of crabs used in the estimation of urinary chloride loss in section C above. Crabs were shaken free of water in a towel, weighed individually, and dried at 95–100° C. Crabs from 50% sea water had a mean water content of 65.6%; those from 10% sea water averaged 65.2%. This constancy is not surprising, since 10% and 50% sea water bound the flattest part of the curve of regulation (Fig. 1).

DISCUSSION

This survey of osmotic performance shows that *Rhithropanopeus harrisi*, as is usual for crabs, produces blood-isotonic urine, the volume of which (24% of body weight per day in an acute exposure to fresh water) is less than that produced by

Carcinus in 40% sea water (Shaw, 1961a; pp. 144–145), more than the production of *Eriocheir* in fresh water (Potts and Parry, 1964; p. 175), and vastly greater than the urine production of *Potamon* in fresh water (Shaw, 1961b). *Rhithropanopeus,* with its rather copious urine production, does not seem to have evolved significantly toward the greatly lowered water turnover rate and low water-permeability reported for *Potamon.*

However, *Rhithropanopeus* exhibits another capability, that of lowering its water-permeability as an adaptive response to a lowered external salinity. This is the first report in crustaceans of a phenomenon that has previously been detected in the brackish-water polychaete annelid *Nereis diversicolor* by Jørgensen and Dales (1957), who showed that worms in fresh water had a water-permeability not greater than 40% of that shown in 11% sea water. Smith (1964) likewise reported a possibly lowered D_2O influx when *Nereis succinea* and *Nereis limnicola* were tested in 5% sea water or fresh water, respectively, although the significance was doubted. The present demonstration of this phenomenon in *Rh. harrisi* raises the probability that environmentally-induced changes in water-permeability of body surfaces may be a more general phenomenon than has hitherto been assumed. It is compatible with the possibility, although it is not a proof, that the apparent low water-permeability of *Potamon niloticus* is an evolutionary refinement of a mechanism present more generally in cancroid Brachyura. It also serves to emphasize that, in discussions of permeability, one must specify to what the permeability applies, since water-permeability may vary independently of permeability to ions (Smith, 1964) or other substances (Leaf, 1965).

On the basis of the D_2O entry rates obtained under natural conditions of stress (10% and 70% sea water are well within the normal salinity variation met by *Rhithropanopeus* in nature) a calculation of the amount of water entering, and presumably available for elimination as urine, may be made by making certain assumptions; the data available seem inadequate for a close correction for back-diffusion. In *ca.* 70% sea water the blood is isotonic to the medium (Fig. 1) and both would have a salt concentration equivalent to 350 mM/L. NaCl (0.70 osmoles). Water concentrations inside and out are equal, namely 55.5–0.7 = 54.8 osmoles, and there should be no *net* osmotic inflow. In this situation we observe (Fig. 4A) that D_2O diffuses in at such a rate that 47.7% of the external concentration is reached in 0.5 hour. The water content of the crab is 65%, so that for a 1-gram crab, if we assume all H_2O in the medium is replaced by D_2O (50.0–0.7 = 49.3 osmoles) the diffusional influx of water (as D_2O) would be expressed by 0.477 × 650 mg./0.5 hours = 620 mg./hr. This influx is balanced by an equal outflux. The influx per mole of external D_2O concentration would be 620 mg. per hr./49.3 moles = 12.6 mg. of water per osmole concentration difference per hour.

If the above 1-gram crab were to be placed suddenly into distilled water (concentration = 55.5 osmoles) there would be a concentration difference of 55.5–54.8 = 0.7 osmoles, producing a *net* water influx of 0.7 × 12.6 = 8.8 mg. per hour per gram of crab. This calculated value of 0.88% of the body weight is not far from the value of 1.05% of body weight per hour estimated in section C as the urine production of crabs adapted to 50% sea water and tested in distilled water. Indeed, a rough calculation of back-diffusion would indicate that diffusional input equals urinary output.

In *ca.* 10% sea water the blood has a salt concentration approximating 240

mM/L. NaCl (.48 osmole), and a water concentration of 55.50–.48 = 55.02 osmoles. The medium has a salt concentration of only $ca.$ 50 mM/L. NaCl (.10 osmole) and a water concentration of $ca.$ 55.4 osmoles. D_2O is observed (Fig. 4A) to diffuse in at such a rate that 36.6% of the external concentration is reached in 0.5 hour. The water content may also be assumed to be 65%. Thus for a 1-gram crab, if we assume all H_2O in the medium to be replaced by D_2O (50.0–0.1 = 49.9 osmoles) the diffusional influx of water (as D_2O) would be $0.366 \times 650/0.5 = 475.8$ mg./hr. The influx per mole of external D_2O concentration would be 475.8 mg. per hr./49.9 osmoles = 9.52 mg. of water (as D_2O) per osmole concentration difference per hour. But in this second instance the crab is not presumed to be in osmotic equilibrium with its medium, there being a water concentration difference of 55.40–55.02 = 0.38 osmole. This should produce a net influx of $0.38 \times 9.52 = 3.62$ mg. of excess water entering per hour in 10% sea water to be disposed of as urine. When such a crab is placed in distilled water the water concentration difference between medium and blood rises to 55.50–55.02 = 0.48 osmole, which should produce a net water influx into a 1-gram crab of 4.60 mg./hr. This calculated value of 0.46% of the body weight is only about half the value of 0.97% of body weight per hour in urine production estimated in section C for crabs adapted to 10% sea water before being tested in distilled water. An estimate allowing roughly for back-diffusion would give a value of 0.54% of body weight, still quite low.

In summary, the diffusional net influx of water (as estimated from D_2O influx) may not be enough to account for the urine produced under conditions when an osmotic influx might be expected to increase the urine flow.

That urinary output of water occurs even in the absence of an "osmotic" inflow resulting from a water-concentration difference has been so well known that it is usually not commented upon. Shaw (1961a, p. 144) has remarked, "It seems quite clear that the water required for urine production in *Carcinus* in full-strength sea water is not taken up osmotically since often no osmotic gradient exists and one must suppose, therefore, that the water is absorbed by some active process . . ." This is quite in harmony with the present observation that urinary output in *Rh. harrisi* may be double the calculated diffusional net water input. The phenomenon is, indeed, rather general, having been first noted in the frog by Hevesy, Hofer and Krogh (1935). Koefoed-Johnson and Ussing (1953) and Ussing (1954) cite other examples and confirm the original findings. Ussing (1954) suggests a simple model combining inner diffusional areas in series with outer narrow channels or "pores" such that, given a small diffusional net influx, a flow is set up in the pores of sufficient velocity to block diffusion in the opposite or outward direction. Such a membrane system would act in respect to diffusion like a one-way valve. It could admit water or D_2O, but since the expected compensating outward diffusion is blocked by the phenomenon of flow in the pores, there would be a greater-than-expected retention of water inside, to be disposed of in the urine. Ussing's concept applied to a crab hyper-regulating in dilute sea water would imply that, instead of an active transport of water as suggested by Shaw, we may have a surface membrane system in which, given a small net inward diffusion of water, the resulting bulk flow inwards through pores or areas behaving like pores results in a net inward movement of water resembling an active transport. Probably it is not that simple, but the epithelium-cuticle system of crustaceans deserves to be examined critically for direct evidence of differential water-

permeability, as Ussing's hypothesis might suggest, or for active water transport as suggested by Shaw.

A further point for discussion is raised by Lockwood (1965, p. 68), who has made the generalization, based on studies of hypotonic urine formation in brackish-water amphipods, that, ". . . the conservation of ions within the body by the production of hypotonic urine is likely to be found to be a common feature of the smaller brackish water crustacea, especially those with a high rate of water turnover." On the basis of this interesting possibility, I have attempted to sample urine from smaller individuals of *Rhithropanopeus,* and am able to state that animals of less than one gram weight show no sign of hypotonicity in their urine, despite their high water turnover. It seems most unlikely that still smaller juvenile members of the population, comparable in size to Lockwood's amphipods, produce hypotonic urine and then give up this physiologically advantageous habit. It is to be noted that Lockwood has shown 80% of the salt loss in *Gammarus duebeni* to be *via* the urine. Probably Lockwood's generalization does not apply to crustaceans which, like *Rhithropanopeus,* suffer the major part of their salt loss *via* the body surface, and his prediction might be better stated as: ". . . the conservation of ions within the body by the production of hypotonic urine is likely to be found in those brackish and fresh water crustaceans which combine a high rate of water turnover with a significant reduction in the salt-permeability of the body surface." Whatever the outcome, Lockwood's stimulating generalization should be tested upon a wide variety of crustaceans.

Summary

1. The osmotic performance of the small cancroid crab *Rhithropanopeus harrisi* (Brachyura) has been surveyed in order to assess the mechanisms mainly responsible for its success in colonizing waters of low salinity.

2. This crab shows hyper-regulation of chloride and osmotic pressure in media up to about 60–70% sea water, and a slight tendency to hypo-regulate in higher salinities.

3. Like other crabs, *Rh. harrisi* maintains a relatively high blood concentration and produces a blood-isotonic urine.

4. Urine production, estimated by an indirect method, approximates 24% of the body weight per day in low salinities, implying a high rate of water turnover.

5. Approximately $\frac{1}{3}$ of the total salt loss is *via* the urine.

6. Inward permeability to water, as judged by D_2O influx rate, is decreased at lower salinities. This mechanism, here demonstrated for the first time in crustaceans, is suggested as being of adaptive significance.

7. Urinary output of water exceeds the diffusional net (osmotic) input of water as calculated by D_2O influx, suggesting the possibility that a differential diffusional permeability to water or some form of non-diffusional water transport may be involved.

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Note added in proof: Since this paper went to press, a pertinent paper by Rudy (1967) has appeared, in which he states that the brackish-water crab *Carcinus maenas* and prawn *Palaemonetes varians* "cannot significantly alter their integumental water permeability." A re-expression of my data in terms approximating Rudy's "H_2O influx constant" (per cent of body water exchanged per hour) and the extrapolation of my weight/uptake curves to give values for 40-gram *Rhithropanopeus* (if such existed) lead me to conclude that no necessary incompatability between our claims exists. *Carcinus* possibly exhibits the same tendency to altered water-permeability as does *Rhithropanopeus,* although perhaps not (on the basis of limited data) to a "significant" extent.

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